Intracoronary injection of bone marrow-derived mononuclear cells early or late after acute myocardial infarction: Effects on global left ventricular function

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Abstract: Background—Intracoronary administration of autologous bone marrow–derived mononuclear cells (BM-MNC) may improve remodeling of the left ventricle (LV) after acute myocardial infarction. The optimal time point of administration of BM-MNC is still uncertain and has rarely been addressed prospectively in randomized clinical trials. Methods and Results—In a multicenter study, we randomized 200 patients with large, successfully reperfused ST-segment elevation myocardial infarction in a 1:1:1 pattern into an open-labeled control and 2 BM-MNC treatment groups. In the BM-MNC groups, cells were administered either early (ie, 5 to 7 days) or late (ie, 3 to 4 weeks) after acute myocardial infarction. Cardiac magnetic resonance imaging was performed at baseline and after 4 months. The primary end point was the change from baseline to 4 months in global LV ejection fraction between the 2 treatment groups and the control group. The absolute change in LV ejection fraction from baseline to 4 months was −0.4±8.8% (mean±SD; P=0.74 versus baseline) in the control group, 1.8±8.4% (P=0.12 versus baseline) in the early group, and 0.8±7.6% (P=0.45 versus baseline) in the late group. The treatment effect of BM-MNC as estimated by ANCOVA was 1.25 (95% confidence interval, −1.83 to 4.32; P=0.42) for the early therapy group and 0.55 (95% confidence interval, −2.61 to 3.71; P=0.73) for the late therapy group. Conclusions—Among patients with ST-segment elevation myocardial infarction and LV dysfunction after successful reperfusion, intracoronary infusion of BM-MNC at either 5 to 7 days or 3 to 4 weeks after acute myocardial infarction did not improve LV function at 4-month follow-up.

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Intracoronary Injection of Bone Marrow Derived Mononuclear Cells, Early or Late after Acute Myocardial Infarction: Effects on Global Left Ventricular Function Four months results of the SWISS-AMI trial

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Abstract:

Background—Intracoronary administration of autologous bone marrow derived mononuclear cells (BM-MNC) may improve remodeling of the left ventricle (LV) after acute myocardial infarction (AMI). The optimal time point of administration of BM-MNC is still uncertain and has rarely been addressed prospectively in randomized clinical trials.

Methods and Results—In a multi-centre study, we randomized 200 patients with large, successfully reperfused ST segment elevation myocardial infarction (STEMI) in a 1:1:1 pattern into an open-labeled control and two BM-MNC treatment groups. In the BM-MNC groups cells were either administered „early", i.e. 5-7 days, or „late", i.e. 3-4 weeks after AMI. Cardiac magnetic resonance imaging was performed at baseline and after 4 months. The primary endpoint was the change from baseline to 4 months in global LV ejection fraction (LVEF) between the two treatment groups and the control group. The absolute change in LVEF from baseline to 4 months was -0.4±8.8% (mean±SD; p= 0.74 vs. baseline) in the control group, 1.8±8.4% (p − 0.12 vs. baseline) in the early group and 0.8±7.6% (p − 0.45 vs. baseline) in the late group. The treatment effect of BM-MNC as estimated by ANCOVA was 1.25 (95% CI -1.83 to 4.32; p = 0.42) for the early and 0.55 (95% CI -2.61 to 3.71; p = 0.73) for the late therapy group.

Conclusions—Among patients with STEMI and LV dysfunction following successful reperfusion, intracoronary infusion of BM-MNC either at 5-7 days or 3-4 weeks after AMI, did not improve LV-function at 4 months follow-up.

Clinical Trial Registration Information—http://www.clinicaltrials.gov; Identifier: NCT00355186.

Key words: bone marrow mononuclear cells, regeneration, myocardial infarction, remodeling, magnetic resonance

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Introduction

Progenitor-cell based therapy using autologous bone marrow as a source has been suggested to improve left ventricular function in patients with acute myocardial infarction (AMI), if administered after successful percutaneous coronary intervention (PCI). Indeed, several published studies using bone marrow derived unselected mononuclear cells (BM-MNC) showed an improvement in global left ventricular (LV) function[1-3] or in regional LV function.[4] Others, however, could not confirm any beneficial effect of cell therapy on LV function.[5,6] Such controversial results could be due to different study designs, different cell isolation protocols potentially leading to differences in cell functionality or be due to the way endpoints have been assessed. Furthermore, timing of cell administration may be an important factor influencing the treatment effect of progenitor-cell based therapy. Indeed, in most of the studies BM-MNC were administered within the first 7 days after AMI. Interestingly, in a pre-specified subgroup analysis of the REPAIR-AMI trial, the beneficial effects of BM-MNC appeared to be more pronounced with later cell application (i.e. 5-7 days).[2] The *Cardiovascular Cell Therapy Network* (CCTRN) performed two trials investigating different time points of cell application: The recently published Time trial[7] compared BM-MNC therapy at 3 days vs. 7 days after AMI, while the Late Time trial[8] tested BM-MNC application 2-3 weeks after AMI against placebo. In neither one of these trials, however, BM-MNC therapy improved LV-function. However, a direct comparison of the effects of early versus late BM-MNC application on LV-function is still lacking. Thus, the SWISS-AMI trial was designed to prospectively investigate the optimal time of BM-MNC administration at two different time points, i.e. “early or 5-7 days or „late“ or 3-4 weeks after AMI.

Methods

Study sample and protocol

The study design with predefined inclusion and exclusion criteria have been previously described.[9] In brief, patients with acute ST elevation myocardial infarction (STEMI) and successful PCI within 24 hours after symptom onset were eligible for enrollment into this multi-center, randomized, controlled trial provided they presented with an estimated left ventricular ejection fraction (LVEF) of < 45% as assessed by an LV-angiogram or transthoracic echocardiography the day of or following the AMI. After giving their informed consent to participate in the study, patients were randomly assigned in a 1:1:1 fashion to one open labeled control and two BM-MNC treatment groups. The control group received best medical management according to current guidelines,[10] including aspirin and clopidgrel or prasugrel, statins, beta-blockers and ACE-inhibitors or ATII-receptor blockers, as well as aldosterone antagonists, if indicated. The „early" BM-MNC treatment group received cells at 5-7 days and the „late" BM-MNC group at 3-4 weeks after primary PCI, on top of best medical management. All patients underwent cardiac magnetic resonance imaging (CMR) at baseline and at 4 months after AMI. The primary hypothesis was that the change in LVEF at 4 months as compared to baseline would be more pronounced in *both* treatment groups compared to control patients. A total of 4 Swiss tertiary centers (University Hospital, Zurich; Bern University Hospital; Cantonal Hospital, Lucerne and Fondazione Cardiocentro Ticino) participated in this trial. The study was conducted in accordance with the declaration of Helsinki and the protocol was approved by the regional ethical Committee of each participating center as well as by the Federal competent authorities (Swissmedic and Federal Office of Public Health).

Bone marrow aspiration and cell processing

Bone marrow aspiration was performed under sterile conditions in patients randomly assigned to the BM-MNC treatment arms with negative serologic testing for hepatitis B, hepatitis C and HIV either 5-7 days or 3-4 weeks after the AMI. The cell processing was done in a centralized, GMP certified facility (Cell Therapy Unit, Cardiocentro Ticino, Lugano, CH). Between 60 and 80 ml of BM were collected from the iliac crest under local anesthesia. Then, 1 ml of a solution containing 1000 IU Heparin was added to each 10ml of bone marrow aspirate to prevent clotting. Then, the aspirate and 20ml of patient‘s serum was sent at room temperature by courier to the cell-processing center. The BM-MNC cell suspension was shipped back to the participating hospital within 24 hours. Shipping protocols as well as the isolation of the mononuclear cell fraction have been performed according to a standard protocol according to previous studies[2,11] with minor modifications as described.[9] Briefly, using density gradient centrifugation, the mononuclear cell fraction was re-suspended in 10ml of serum free medium added with 20% of autologous serum without adding any further heparin. An aliquot of cell suspension has been utilized for fluorescence-activated cell sorting (FACS) analysis using fluorochrome-conjugated antibodies against anti-human CD34 and CD133; cell viability was assessed by 7-AAD cell uptake and sterility by Bact/Alert rapid method. Release criteria of the BM-MNC were product sterility, a cell count between $5x10^7$ and $5x10^8$ and cell viability of ≥80%. Migration capacity of BM-MNC was measured in a modified Boyden chamber as previously described.[12] It was expressed as percentage of mononuclear cells able to *actively* cross a membrane coated with extracellular matrix proteins and using an invasion index, indicating the ratio between the latter and the percentage of cells *passively* crossing the same membrane when uncoated.

Intracoronary infusion of the BM-MNC

After obtaining arterial access (either via common femoral artery or the radial artery), patients

received 5.000 IU of heparin i.v. Then, an over-the-wire balloon catheter was advanced via a guiding catheter in the segment of the former infarct related vessel containing the stent. After inflation of the balloon with low pressure (2-4 bar) within the stented segment to obtain total occlusion of the vessel, BM-MNC were infused within 3 minutes to allow for adhesion and transmigration of the infused cells through the endothelium („stop-flow technique“). This maneuver was repeated three times to allow for infusion of the total of 9 ml progenitor cell suspension, interrupted by 5 minutes of reflow by deflating the balloon to minimize extensive ischemia and pain. Finally, a coronary angiography was repeated to ascertain vessel patency.

Periprocedural safety of the BM-MNC infusion has been monitored by assessment of serum cardiac enzymes including cardiac troponine the day after the intervention. Periprocedural myocardial infarction was defined as previously described.[13]

Cardiac magnetic resonance imaging (CMR)

Cardiac imaging was performed using 1.5-Tesla clinical MR systems. Dedicated cardiac phased-array receiver coils were used for signal reception. Patients underwent CMR studies at baseline, i.e. during the hospitalization for the AMI and at 4 months of follow-up. Following localizer acquisitions, the CMR studies assessed functional imaging of the LV by means of standard ECG-triggered steady-state free precession acquisitions during repetitive breath-holds in 3 long-axis orientations and in contiguous short-axis orientation covering the entire LV. In the second part of the CMR examination, scar imaging has been performed after administration of a bolus of a conventional extracellular gadolinium-chelates contrast medium at a dose of 0.20 mmol of per kilogram of body weight by using an inversion-recovery (IR) fast gradient echo imaging sequence.[14,15] After determination of the inversion time nulling for normal myocardium, scar imaging was performed 20 minutes after administration of contrast medium in identical locations

as functional data were acquired.

CMR data analysis was performed in a core laboratory (University Hospital Zurich/CH) using dedicated cardiac analysis software (GTVolume, Gyrotools Ltd, Zurich/CH). LV end-diastolic (LV-EDV) and end-systolic (LV-ESV) volumes, LVEF and LV-mass have been quantified for the assessment of the primary endpoint (change of LVEF) and for assessment of ventricular remodeling over time in the 3 study groups.

Regional wall motion was assessed measuring systolic and diastolic wall thickness in each of the 6 segments of all acquired slices and thickening of each segment was calculated subtracting systolic and diastolic values. A global thickening index was elaborated accounting for the median of all values of each segment. The same was repeated in the infarct territory accounting only for the infarct related segments.

The extent of microvascular obstruction (MVO) delineated as dark areas in the core of the necrotic zone in the late enhancement images were quantified by contouring manually the dark core areas. Scar mass and tissue with MVO were assessed in grams (g) and milliliters (ml) (data not shown) and as a percentage of LV-mass and of scar mass, respectively.

Changes over time (i.e. 4 months versus baseline) of volumetric, functional and scar parameters were compared between the groups to assess the influence of treatment on global and regional systolic function, left ventricular remodeling and scar mass.

Endpoints

The primary endpoint was the absolute change in global LVEF from baseline compared to 4 months follow-up between the control group and both BM-MNC therapy groups, respectively.

Secondary endpoints comprised the change in global LVEF from baseline to 4 months between control and a combined therapy group, as wells as changes in LV-EDV and LV-ESV,

change in infarct size, change in the proportion of scar mass to total LV mass and changes in global and regional myocardial thickening. In order to identify predictive markers, we analyzed treatment-marker interactions of the primary endpoint with infarct size, microvascular obstruction, baseline nt-pro brain natriuretic peptide (nt-proBNP) and the time from the onset of chest pain to successful reperfusion therapy of the AMI. Pre-specified clinical end points were analyzed including major adverse events (defined as all-cause death, recurrence of myocardial infarction, any coronary revascularization procedure or rehospitalization for heart failure; all at 4 months) and time from STEMI to the occurrence of such events at 4 months. Only the first event for each patient was included in the analysis.

Statistical analysis

In general, median and interquartile range (IQR) was presented for continuous variables. If criteria for normal distribution were fulfilled, variables were presented as mean and standard deviation (SD). Nominal variables were summarized in terms of frequencies and percentages.

For the analysis of the primary endpoint, we performed analysis of covariance (ANCOVA) including LVEF values at 4 months as dependent variables and the associated baseline values and the factor treatment as independent variables. Estimates of the treatment effect are presented together with the 95% confidence interval. Since comparisons of both experimental groups to control were considered as primary endpoint, p-values were compared to the Bonferroni-corrected significance level $\alpha = 0.025$.

The same analysis as for the primary was repeated for the secondary endpoints (only ANCOVA analyses are shown). For the analyses of the primary and secondary endpoints, only matched CMR data (baseline and 4 months) was accounted.

As additional secondary endpoint, aiming to find easy interpretable predictive markers for treatment success prespecified continuous baseline markers were thus split at the median (in one case at a manually chosen cutoff). With this new variable the p-value for the interaction was then computed in an ANCOVA model with LVEF at 4 months as dependent variable and the associated baseline values, the factor treatment, the marker under consideration discretized at cutoff, and treatment - marker interaction as independent variables.

To compute regressions for LVEF at 4 months follow up, further ANCOVA analysis involving adjustment for baseline LVEF, was performed. A total of 21 binary, 7 nominal, and 40 continuous explanatory variables have been separately analyzed.

Binary endpoints were compared between groups using Chi-square or Fisher's exact test, depending on whether expected cell frequencies were < 5 or not. For continuous outcomes independent samples t- or Wilcoxon test were used.

Major adverse cardiac events (MACE) are defined as the occurrence of one of the predefined clinical scenarios (death, myocardial infarction, coronary revascularization, or rehospitalization for heart failure) and were compared between groups at 4 months.

As for the secondary endpoints multiple comparisons were performed, the analysis of the results shall be designated 'exploratory'. p-values and 95% confidence intervals are therefore shown without further Bonferroni correction . All computations were done with R (R Development Core Team, 2012).

Sample size / power calculation

The study has been powered for evaluation of the primary endpoint depending on the presence of paired CMR data, assessed either at baseline and at 4 months follow up.

As previously described[9] and according to recent studies,[1,2,16] in planning sample size we

assumed a difference between LVEF improvements from baseline to 4 months of $\delta = 3.5\%$ with a standard deviation of 6% between control and both treatment groups. A sample size of n = 58 per group was therefore needed to detect such difference with a power of 80% for Bonferroni-corrected significance level of $\alpha = 0.025$. Accounting for a dropout rate of 10% we planned to include n = 64 per arm and 192 for the entire trial. All computations were done with R (R Development Core Team, 2012).

Results

Patient enrollment and baseline characteristics

Between October 2006 and January 2012, in the 4 Swiss cardiovascular centers, a total of 200 patients gave informed consent to participate in the trial. Of those, 66 patients have been randomized in the early BM-MNC treatment group and 67 patients in the control and late treatment group, respectively. As the dropout rate was higher than expected, the initially planned sample size had to be increased from 192 to 200 patients, allowing to attain at least for two study arms with the necessary sample size. Particularly within the "late" treatment group dropout was more common. Patient flow from randomization until 4 months follow up is shown in **Figure 1**. There was no statistically significant difference in baseline characteristics between the three groups apart from a higher age and a lower percentage of smokers in the „late" treatment group as well as a higher baseline LVEF in the control group **(Table 1).** Baseline CMR was performed at a median of 6 (IQR 4) days after the AMI.

Patients, withdrawing informed consent or unwilling to undergo repeat CMR examination, were generally older and had higher values of maximal creatine kinase at baseline, especially in the "early" treatment group, but not in the "late" therapy group, were the highest

drop-out occurred.

Characteristics of index myocardial infarction

Overall, 92% of the patients had anterior STEMI due to LAD occlusion and the median time from onset of chest pain to reperfusion therapy was 4.5 hours (IQR 5.25). 76% of the patients were treated with glycoprotein IIb/IIIa inhibitors or bivalirudin, 78% received a drug eluting stent with a median diameter of 3.5mm (IQR 0.5). The median of maximal creatine kinase (CKmax) plasma levels was 3919 U/l (IQR 3664), and baseline LVEF, as assessed by CMR at a median of 6 (IQR 4) days after the index AMI, was 37.4% (IQR 13.2).

Characteristics of BM-MNC

A total of 153 (119) x 10^6 nucleated cells have been infused. Besides an impurity of granulocytes, the mononuclear fraction consisted mainly of lymphocytes and monocytes and to small part of functional hematopoietic, endothelial/angiogenic and mesenchymal precursors (**supplementary table S1**). Between 1% and 1.3% of the cells were $CD34^+$ cells. The entire cell processing data are shown in **Table 2.** There was no significant difference between early and late therapy group except for a higher percentage of $CD\ 34^+$ cells in the late therapy group. For a subset of patients (n = 59) we performed functional test; the median (IQR) percentage of MNC exhibiting invasion capacity, was overall 29 (19) % and the overall invasion index was 49 (25) % without difference between early and late BM-MNC treatment group.

Assessment of left ventricular function and remodeling at baseline and 4 months follow up

Primary Endpoint

The mean (SD) absolute change in LVEF at 4 months was -0.4 (8.8) % in the control group, 1.8 (8.4) % in the „early“ group and 0.8 (7.6) in the „late“ group (**Figure 2**). Adjusting for baseline LVEF using an ANCOVA model, the estimated treatment effect averaged 1.25 (95% CI -1.83 to

4.32; p=0.42 vs. control) for the early therapy group and 0.55 (95% CI -2.61 to 3.71; p = 0.73 vs. control) for the late therapy group (**Table 3**).

Secondary Endpoints

Combining the "early" and "late" to a common BM-MNC therapy group (n = 107), the estimated treatment effect was 0.85 (95%CI -1.75 to 3.44; p = 0.52 vs. control).

Negative remodeling occurred in all 3 groups. The median (IQR) LVEDV increased from 154 (44) to 175 (76) ml in the control group, from 153 (49) to 185 (64) ml for the early and from 149 (47) to 165 (73) ml in the late treatment group. Likewise, LVESV increased from 94 (35) to 114 (68) ml in the control group, from 94 (41) to 105 (50) ml in the early and from 97 (38) to 103 (54) ml in the late treatment group. Only for the late treatment group, ANCOVA testing revealed less negative remodeling (estimated treatment effect -14.86; 95% CI -28.98 to -0.74; p = 0.04 for LVEDV and -10.73; 95% CI -22.86 to 1.39; p = 0.08 for LVESV). In all groups, total scar mass uniformly decreased by more than 10g corresponding to a 4-5% decrease in the proportion of myocardial scar with respect to the entire myocardial mass.

Global LV thickening slightly decreased in all 3 groups. In contrast, myocardial thickening in the infarct-related segments only showed negligible changes. For both parameters no treatment related between-group difference was found. The entire results of CMR analyses at baseline and 4 months as wells as ANCOVA testing are shown in **Table 3**.

In a prespecified analysis for predictors of BM-MNC treatment effect, the entire patient sample was split according to the median value of selected variables. No significant interaction was found for infarct size and for microvascular obstruction (splitted at the median). However, an interaction with the treatment effect of BM-MNC was found for *time from onset of chest pain to successful reperfusion therapy* (p = 0.0455 for early vs. control; p = 0.0035 for late vs.

control) as well as for the *baseline value of nt-proBNP* (p = 0.023 for early vs. control; p = 0.0097 for late vs. control). Interestingly, early-reperfused patients (below the median value of 4.5 hours) demonstrated an importantly higher treatment effect of BM-MNC (**Figure 3**), either injected early or late after AMI, as compared to controls (estimated treatment effect of 6.31; 95% CI 0.13 to 12.48; p = 0.046 for the early and of 9.17; 95% CI 3.08 to 15.26; p = 0.004 for the late BM-MNC therapy group). Likewise, patients with higher nt-proBNP levels at baseline (above the median value of 1437 ng/l) demonstrated a higher effect of BM-MNC treatment in both therapy groups as compared to controls (estimated treatment effect of 7.1; 95% CI 1.00 to 13.20; p = 0.023 for the early and of 9.02; 95% CI 2.24 to 15.79; p = 0.01 for the late BM-MNC therapy group). As for the absence or presence of microvascular obstruction at baseline there were inconsistent results showing significant interaction for the late, but not for the early therapy group.

Overall, regression analysis (**supplementary table S2**) did not show an influence of cell-related parameters (total number of mononuclear cells, total number of $CD34^+$ and $CD133^+$ cells, proportion of $CD34^+$ and $CD133^+$ cells and migratory capacity) or age on LVEF at 4 months, adjusted for baseline LVEF.

Clinical follow up and events

At 4 months, more than 75% of the patients were in NYHA class I and more than 92% in CCS class I. There was no difference between groups (see **supplementary Table S3**). The clinical event rates are shown in **Table 4.** Events occurring between randomization and cell therapy and for the cumulative 4 months rates are presented separately. Overall mortality at 4 months was low (2%) despite the fact that high-risk patients with large AMIs were enrolled. No death has been noticed in the control group, whereas one patient (1.7%) died in the late and 3 (4.6%) in the

early treatment group. Of note however, two death (one of each group) occurred between randomization and a scheduled BM-MNC treatment. There was no significant difference in the frequency of isolated serious adverse events at 4 months between the three groups, neither of the prespecified, combined clinical endpoint of death, recurrence of myocardial infarction, repeated coronary revascularization nor of rehospitalization for heart failure.

Discussion

The Swiss-AMI trial, to the best of our knowledge, is the first randomized controlled clinical study, which addresses either an early, as well as a late time point of BM-MNC administration after AMI. Both therapy groups were compared to a control arm in a unique trial design, assuming an equal treatment effect. The study design and power calculation was based the knowledge and data available in 2006 for the calculation of adequate sample size. Furthermore, standardized state-of-the-art cell processing and the best imaging modality (i.e. CMR) were used to test this hypothesis. Surprisingly, the results of our study do not confirm any significant improvement of global LV-function at 4 months follow-up, neither with early (5-7 days) nor with late (3-4 weeks) application of BM-MNC after a first and rather large AMI. Accordingly, the between-group mean difference between early cell therapy and control was 2.1% (SD >8%), far away from the 3.5% improvement (SD 6%) that was assumed for sample size calculation. For none of the secondary endpoints a consistent benefit of cell therapy for both the early and late therapy group could be found.

Among the 5 potential predictors for a significant treatment effect analyzed we identified nt-proBNP levels above the median and the time from the onset of chest pain to reperfusion therapy below the median of 4.5 hours as the most promising. A potential explanation for the

latter finding may be that early reperfusion after AMI leads to lower infarct transmurality but not to a lower infarct size or to less microvascular obstruction, as shown previously.[17] In line with the results of a smaller trial,[18] we suggest that in patients with complete transmural scar, BM-MNC treatment may be less beneficial. Further analyses of the 12 months CMR data, currently under way, will reveal whether remodeling is favorably affected by BM-MNC therapy in the long run.

Our study fulfilled several prerequisites for a properly designed randomized clinical trial testing progenitor-cell based therapy. First, we managed to enroll mainly patients with large myocardial infarction, as demonstrated by an overall median LVEF of 37% and a rather high peak plasma level of CK. This was not the case in most of the previous trials.[1-3] It is of note that the size of AMI has been shown to favor beneficial effects of cell therapy in subgroup analyses.[2,19] Thus the patient population of the Swiss-AMI trial was uniquely suited to show a potential benefit of cell therapy. Second, the assessment of cardiac function and infarct parameters was based on CMR, which is currently considered the gold standard for the analysis of global and regional LV-function[20,21] and is recognized as one of the most accurate techniques to quantify necrotic or fibrous tissue.[22-24] Moreover, the entire analysis was performed in a CMR core-lab, blinded to the treatment assignment of the patients enrolled. In line with previous studies that used CMR to assess LV function, the changes in LVEF and remodeling were overall small or negligible.[4-6,8,19,25] Furthermore, for the interpretation the data, the time point of the baseline CMR study may be important. To account for the early improvement in LV-function due to the recovery of stunned myocardium, we performed the baseline examination at day 6 after AMI. This could explain the absence of improvement of LVEF in the control group from baseline to the 4 months follow-up, which was notable for instance in the REPAIR-AMI study.[2]

Third, the processing of BM-MNC was performed by an experienced[26] and certified core-laboratory, using a standardized protocol as used in previous trials.[2] Cell potency was assessed *in vitro* as described[12] confirming appropriate BM-MNC viability. Furthermore, *in vivo* experiments using the mouse model of myocardial infarction as recently described by us are currently ongoing.[27] Heparin, which potentially may abrogate the migration capacity of BM-MNC,[28] has not been added directly to the cells. As many of the research groups working in the field, we choose unselected MNC as treatment agents, because they have been proven to be safe in previous clinical studies and are easy to obtain without complex purification and cultivation steps. Furthermore, selected MNC of any type have never been shown to be superior to unselected MNC in terms of neoangiogenesis if confronted directly in clinical trials. Of note, however, that recent studies using selected, bone marrow derived mesenchymal stem cells (MSC) showed a promising increase in LVEF shortly after STEMI, compared with placebo.[29] In addition, CD34+ cells have been shown to successfully reduce refractory angina if injected directly in the ischemic myocardium.[30]

BM-MNC generally contain at a small amount of progenitors cells including hematopoietic stem cells, MSC,[31] endothelial progenitor cells,[32] multipotent adult mesenchymal progenitors[33] and very small numbers of embryonic-like stem cells.[34] Although it has never been clarified which subsets of such progenitor cells may be responsible for beneficial effects of BM-MNC in AMI, their mechanism of action is likely to involve paracrine effects mediated by cells contained in unselected BM-MNC, supporting the use of unselected over selected BM-MNC. Of note, in the Swiss-AMI trial the proportion of $CD34^+$ cells, which are thought to be particularly important for neo-angiogenesis,[35] was 1% in the early and 1.3% in the late therapy group respectively and thus comparable to previous trials. The total number of injected nucleated cells

(overall median of 153 million cells) was lower than in REPAIR AMI or BOOST,[1,2] but similar to LATE-TIME trial[8] and Time trial.[7] According to published data,[36] doses of >150 million of injected mononuclear cells (as was used in the Swiss-AMI trial) have been shown to be sufficient to modify LV function. In addition, a clear relationship between total number of injected mononuclear cells or the $CD34^+$ cells and clinical efficacy has until now never been proven in prospective studies.

There are also several limitations of the study. The long duration between enrollment of the first and the last patients may lead to imbalances in the management of STEMI, as during the study period new antiplatelet agents have been introduced (such as bivalirudin and prasugrel). In addition, the drop out differed importantly between groups. Interestingly, dropout rate was the lowest in the early treatment group, in which the patients received BM-MNC treatment during the initial hospitalization for AMI, while in the late treatment group it was much higher than expected. This may have lead to a certain selection bias. Furthermore, baseline LVEF was somewhat higher in the control group compared to both treatment groups. However, accounting for the results of the subgroup analysis of two previous studies,[2,19] this may have rather disadvantaged the results of the control group and thus cannot account for the overall disappointing results. Furthermore, in the analysis of the primary endpoint, the results have been adjusted for baseline LVEF.

Besides the primary endpoint analysis, we performed a large number of statistical tests involving the secondary endpoints, which may increase the overall probability of a type I error. The results of the subgroup analysis have to be therefore strictly considered as "hypothesis generating". Finally, considering the promising results of recent studies, using selected cells,[29,30] like CD34+ cells or MSC, the lack of cell selection in our study may be also seen as important

limitation.

The results of the Swiss-AMI trial may further cool down the euphoria, which initially accompanied the clinical application of progenitor-cell based research. In the last decade, intracoronary injection of BM-MNC has been tested in several randomized controlled clinical trials and improvement in global LV-function has been reported by some,[1-3] but not all of the investigators.[4-8,19] Especially the results of the Repair-AMI trial[2] were considerably promising and encouraging. Potential factors that may have influenced these controversial results on LV remodeling may relate to cell functionality[37] which has shown to be abrogated by heparin[28] and which may be impaired in cardiovascular patients,[27] selection of patients, in particular differences in baseline LVEF and infarct size[2,19] and the choice for the optimal time point of cell application. For the latter, initial recommendations were based on the work of an expert committee.[38] however in the absence of any prospective data.

Simultaneously with the Swiss-AMI trial, the *Cardiovascular Cell Therapy Network* (CCTRN) started two similar cell therapy trials to prospectively address different time points of cell administration. [7,8] Surprisingly, none of these trials could confirm relevant efficacy of BM-MNC to improve LV-function at none of the tested time points. BM-MNC injection at 2-3 weeks after AMI failed to demonstrate improvement in LV-function in the Late-Time trial compared to placebo.[8] Although an early treatment group was missing in the Late-Time trial, their results are in agreement with the results of the "late therapy" group of Swiss-AMI. In the latter trial, injection at 5-7 days after AMI showed a small, non-significant improvement in LVEF, comparable to the results of the respective subgroup of the Time trial.[7] Taking into consideration all randomized clinical trials, using CMR to assess global and regional LV-function, the effect of BM-MNC on LVEF seems to be rather marginal as shown in a meta-analysis.[36]

In conclusion, the Swiss-AMI trial in patients with STEMI and LV dysfunction following successful reperfusion therapy by primary PCI and intracoronary infusion of BM-MNC either 5-7 days or 3-4 weeks after AMI, we did not find improved LV-function at 4 months. The question whether the measurement of LVEF is the proper end point to assess the clinical utility of cell-based therapy remains open[25] and will await the results of upcoming large outcome trials.

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Table 1. Baseline characteristics of the included patients

	Control (n = 67)	Early (n = 65)	Late (n = 63)	p-value
Age – years (median; IQR)	56 (14.5)	55 (15)	62 (15)	0.70* / 0.06‡
BMI - kg/m2 (median; IQR)	26.7 (4.4)	27.0 (6.1)	27.0 (4.4)	0.92* / 0.89‡
Male gender - %	83.6	86.2	82.5	0.18* / 1.00‡
Hypertension - %	43.3	49.2	38.7	0.60* / 0.72‡
Hyperlipidemia - %	44.8	40.0	41.9	0.60* / 0.86‡
Diabetes - %	17.9	7.7	9.7	0.12* / 0.21‡
Smoking (active/previous) - %	62.7	67.7	40.3	0.60* / 0.01‡
Familiary history of CAD - %	35.8	26.1	24.2	0.26* / 0.18‡
1 / 2 / 3 vessel disease %	64/21/15	54/32/14	57/27/16	0.34* / 0.73‡
Previous PCI before AMI - %	3.0	3.1	1.6	1.00* / 1.00‡
Infarct treatment				
Primary PCI – %	94.0	98.5	100.0	0.37* / 0.12‡
Concomitant PCI other than infarct related artery – %	18.2	12.3	11.1	0.47* / 0.32‡
Infarct vessel LAD/LCX/RCA -%	89/3/8	95/2/3	92/3/5	0.51* / 0.89‡
Pain to revascularization time (h)	4.5 (5)	4.8 (5.4)	4.0 (4.8)	0.57* / 0.53‡
Stent diameter (mm)	3.5 (0.5)	3.0 (0.5)	3.5 (0.5)	0.73* / 0.89‡
Drug eluting stent – %	71.6	80.0	81.0	0.31* / 0.23‡
TIMI flow before PCI	0 (0)	0 (0)	0 (0)	0.31* / 0.87‡
TIMI flow after PCI	3 (0)	3 (0)	3 (0)	0.94* / 0.81‡
Use of Glycoprotein IIb/IIIa inhibitors/bivalirudin -%	71.7	78.5	78.1	0.88* / 0.20‡
Maximal creatin kinase - U/l (median; IQR)	3671 (3685)	4314 (3561)	3436 (3813)	0.22* / 0.78‡
nt-pro BNP - ng/l (median; IQR)	1103 (1848)	1450 (1442)	1581 (1912)	0.15* / 0.10‡
Intra aortic balloon pump / other assist device - %	16.4	15.6	22.6	1.00* / 0.18‡
CMR characteristics of the LV				
LVEF – % (median;IQR)	39.6 (11.2)	34.6 (16.1)	35.6 (11.2)	0.07* / 0.03‡
LVEDV – ml (median;IQR)	154 (44)	153 (49)	149 (47)	0.89* / 0.96‡
LVESV – ml (median;IQR)	94 (35)	94 (41)	97 (38)	0.54* / 0.41‡
Scar mass – g (median;IQR)	39.1 (37.2)	37.7 (32.1)	33.9 (24.2)	0.94* / 0.21‡
Myocardial scar – % (median;IQR)	28.3 (16.3)	28.1 (16.2)	26.6 (15.9)	0.78* / 0.53‡
MVO – g (median;IQR)	0.27 (1.55)	1.08 (3.00)	0.64 (2.49)	0.11* / 0.51‡
Medication at discharge / after 4 months –%				
Aspirin	98.5 / 98.4	98.4 / 98.4	98.3 / 98.2	1.00 / 1.00* 1.00 / 1.00‡
Clopidogrel or Prasugrel	100 / 100	100 / 100	100 / 98.2	1.00 / 1.00* 1.00 / 0.84‡
ACE-inhibitor or ATII receptor blocker	95.5 / 100	100 / 100	96.6 / 98.2	1.00 / 1.00* 1.00 / 0.84‡
Beta-blocker	86.4 / 85.2	91.9 / 88.5	93.2 / 92.6	0.40 / 0.79* 0.25 / 0.25‡
Aldosterone antagonist	12.1 / 11.5	12.9 / 14.8	15.2 / 9.3	1.00 / 0.79* 0.79 / 0.77‡
Statin	97.0 / 98.4	100 / 95.1	98.3 / 100	0.50 / 0.62* 1.00 / 1.00‡

IQR: interquartile range; BMI: body mass index; CAD: Coronary artery disease; PCI: Percutaneous coronary intervention; AMI: acute myocardial infarction; LAD: left anterior descending coronary artery; LCX: left circumflex artery; RCA right coronary artery; TIMI: Thrombolysis in Myocardial Infarction; ACE: angiotensin-converting–enzyme; AT: angiotensin.

*: p value control vs. early

‡: p value control vs. late

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Table 2. Characteristics of BM-MNC and cell treatment

	Early (n = 62)	**Late (n = 52)**	**p-value** (between group difference)
Cell characteristics (Median, IQR)			
BM aspiration volume (ml)	65 (15)	70 (15)	0.30
Total nucleated cells (10^6 cells)	159.7 (125.8)	139.5 (120.5)	0.18
Viability (%)	93.6 (5.55)	93.33 (6.60)	0.98
% CD 34+ cells	1.02 (0.72)	1.31 (0.97)	0.01 #
Total CD 34+ cells (10^6 cells)	1.6 (1.69)	1.45 (2.43)	0.68
% CD 34+/133+ cells	0.81 (0.78)	0.87 (0.97)	0.34
Total CD 34+/133+ cells (10^6 cells)	0.96 (1.46)	0.92 (2.06)	0.77
% Invasion	33 (18) *	26.5 (16.5) **	0.18
Invasion index	50.88 (24.38)*	45.64 (22.10) **	0.21
Timing of BM-MNC treatment			
Days after AMI	6 (2)	24 (7)	NA

BM: bone marrow; % invasion: percentage of total nucleated cells showing invasion capacity
#: estimated Wilcoxon effect -0.31, 95% CI -0.56,-0-07)
*: n = 29
**: n = 30

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Table 3. Analysis of covariance (ANCOVA) of the Primary and the Secondary Endpoints, as assessed by CMR, at baseline and at 4 months follow-up

Variable		Group			Difference treatment groups – control group #	
		Control	*Early*	*Late*	*Estimate (95%CI)*	*p-value*
Primary Endpoint †						
Global LVEF - %						
baseline	Median (IQR)	39.6 (11.2)	34.6 (16.1)	35.6 (11.2)	**1.25**	0.42 *
	Mean (SD)	40.0 (9.9)	36.5 (9.9)	36.3 (8.2)	**(-1.83 to 4.32) ***	
4 months	Median (IQR)	38.7 (17.3)	40.1 (14.8)	37.8 (11.7)	**0.55**	0.73 ‡
	Mean (SD)	39.6 (12.0)	37.9 (10.3)	37.4 (9.7)	**(-2.61 to 3.71) ‡**	
Secondary Endpoints †						
LVEDV – ml						
baseline	Median (IQR)	154 (44)	153 (49)	149 (47)	**0.92**	0.89 *
	Mean (SD)	153 (38)	156 (41)	157 (37)	**(-11.95 to 13.78) ***	
4 months	Median (IQR)	175 (76)	185 (64)	165 (73)	**-14.86**	0.04 ‡
	Mean (SD)	180 (52)	183 (55)	167 (45)	**(-28.98 to -0.74) ‡**	
LVESV – ml						
baseline	Median (IQR)	94 (35)	94 (41)	97 (38)	**-2.06**	0.72 *
	Mean (SD)	94 (33)	100 (36)	100 (29)	**(-13.5 to 9.38) ***	
4 months	Median (IQR)	114 (68)	105 (50)	103 (54)	**-10.73**	0.08 ‡
	Mean (SD)	112 (46)	117 (51)	107 (40)	**(-22.86 to 1.39) ‡**	
Mass myocardial scar - g						
baseline	Median (IQR)	39.1 (37.2)	37.7 (32.1)	33.9 (24.2)	**-0.43**	0.86 *
	Mean (SD)	45.3 (28.0)	44.0 (22.3)	38.5 (22.5)	**(-5.17 to 4.31) ***	
4 months	Median (IQR)	27.8 (17.2)	25.3 (19.7)	21.9 (14.4)	**-2.99**	0.20 ‡
	Mean (SD)	29.2 (15.7)	28.9 (15.7)	24.3 (11.1)	**(-7.64 to 1.66) ‡**	
Myocardial scar - %						
baseline	Median (IQR)	28.3 (16.3)	28.1 (16.2)	26.6 (15.9)	**-1.15**	0.45 *
	Mean (SD)	29.1 (13.1)	28.2 (11.7)	28.1 (11.9)	**(-4.19 to 1.89) ***	
4 months	Median (IQR)	24.3 (14.5)	22.2 (12.0)	22.9 (11.1)	**-1.37**	0.41 ‡
	Mean (SD)	23.9 (10.4)	22.7 (9.4)	22.4 (8.5)	**(-4.67 to 1.94) ‡**	
Global wall thickening (average of all segments) – mm						
baseline	Median (IQR)	8.7 (2.7)	9.3 (3.5)	8.6 (3.1)	**-0.21**	0.34 *
	Mean (SD)	9.1 (2.3)	9.5 (2.3)	8.7 (2.2	**(-0.66 to 0.23) ***	
4 months	Median (IQR)	7.1 (1.9)	6.9 (1.4)	6.6 (2.0)	**-0.10**	0.68 ‡
	Mean (SD)	7.2 (1.3)	7.1 (1.3)	7.0 (1.5)	**(-0.60 to 0.39) ‡**	
Wall thickening in the infarct zone (average of all infarcted segments) – mm						
baseline	Median (IQR)	1.6 (1.2)	1.7 (2.0)	1.7 (1.6)	**-0.075**	0.72 *
	Mean (SD)	1.7 (1.2)	1.6 (1.2)	1.9 (1.2)	**(-0.49 to 0.34) ***	
4 months	Median (IQR)	2.0 (1.8)	2.1 (1.4)	2.1 (1.8)	**0.064**	0.79 ‡
	Mean (SD)	2.2 (1.5)	2.2 (1.2)	2.2 (1.4)	**(-0.41 to 0.54) ‡**	

†: Descriptive statistic (median (IQR) and mean (SD)) of all available parameters at a given time

#: Estimates of the regression coefficient of the treatment from the ANCOVA models.

*: p-value control vs. early

‡: p-value control vs. late

Example for the analysis of the primary endpoint (control group = reference level; Intercept = 9.37; LVEF baseline = 0.76): For the early group patients have on average a LVEF value at 4 months that is 9.37 + 0.76 x (the patient's baseline LVEF) + 1.25. For a patient seen with a mean LVEF of 38%, a LVEF at four months of 9.37 + 0.76 x 38 + 1.25 = 39.5. Confidence intervals and p-value are adjusted for baseline LVEF.

Table 4. Clinical events during follow up

	Control	Early	Late	p-value
Events between randomization and therapy				
Death	0	1 (3.1%)	1 (1.7%)	0.24* / 0.48‡
Events at 4 months follow up (cumulative)				
Death	0	3 (4.8%)	1 (1.7%)	0.24* / 0.48‡
Myocardial infarction	1 (1.6%)	1 (1.6%)	0	1.00* / 1.00‡
Rehospitalization for heart failure	2 (3.2%)	0	2 (3.6%)	0.50* / 1.00‡
Revascularization	3 (4.8%)	3 (4.9%)	2 (3.6%)	1.00* / 1.00‡
Cerebral infarction	1 (1.6%)	1 (1.7%)	0	1.00* / 1.00‡
Combined events				
Death, myocardial infarction, revascularization, rehospitalization for heart failure	4 (6.4%)	5 (7.9%)	5 (8.8%)	1.00* / 0.74‡
Death, myocardial infarction, revascularization, rehospitalization for heart failure, stroke	4 (6.4%)	6 (9.5%)	5 (8.8%)	0.74* / 0.74‡

*: p value control vs. early
‡: p value control vs. late

Figure Legends:

Figure 1. Flow diagram of patient enrolment until 4 months follow-up. STEMI denotes *ST elevation myocardial infarction*; PIC denotes *patient informed consent*

Figure 2. Descriptive statistic (boxplot) of the mean of the differences between LVEF at 4 months and LVEF at baseline for control vs. early and control vs. late BM-MNC treatment.

Figure 3. Descriptive statistic (boxplot) of the mean of the differences between LVEF at 4 months and LVEF at baseline depending on time from onset of pain to reperfusion therapy (control vs. early BM-MNC group (left) and for control vs. late BM-MNC group (right)).

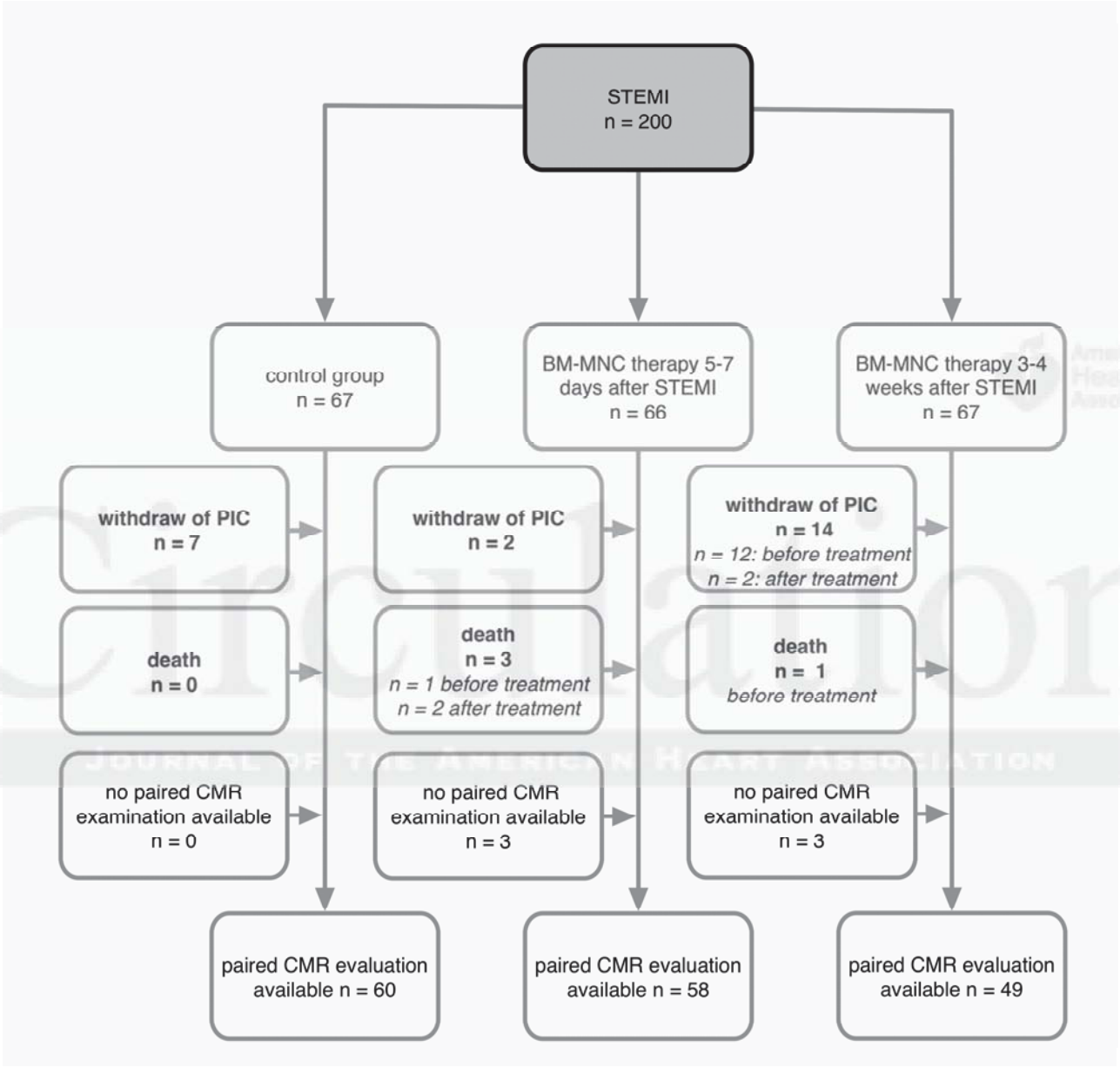
STEMI
n = 200
control group
n = 67
BM-MNC therapy 5-7 days after STEMI
n = 66
BM-MNC therapy 3-4 weeks after STEMI
n = 67
withdraw of PIC
n = 7
withdraw of PIC
n = 2
withdraw of PIC
n = 14
n = 12: before treatment
n = 2: after treatment
death
n = 0
death
n = 3
n = 1 before treatment
n = 2 after treatment
death
n = 1
before treatment
no paired CMR examination available
n = 0
no paired CMR examination available
n = 3
no paired CMR examination available
n = 3
paired CMR evaluation available n = 60
paired CMR evaluation available n = 58
paired CMR evaluation available n = 49

Figure 1

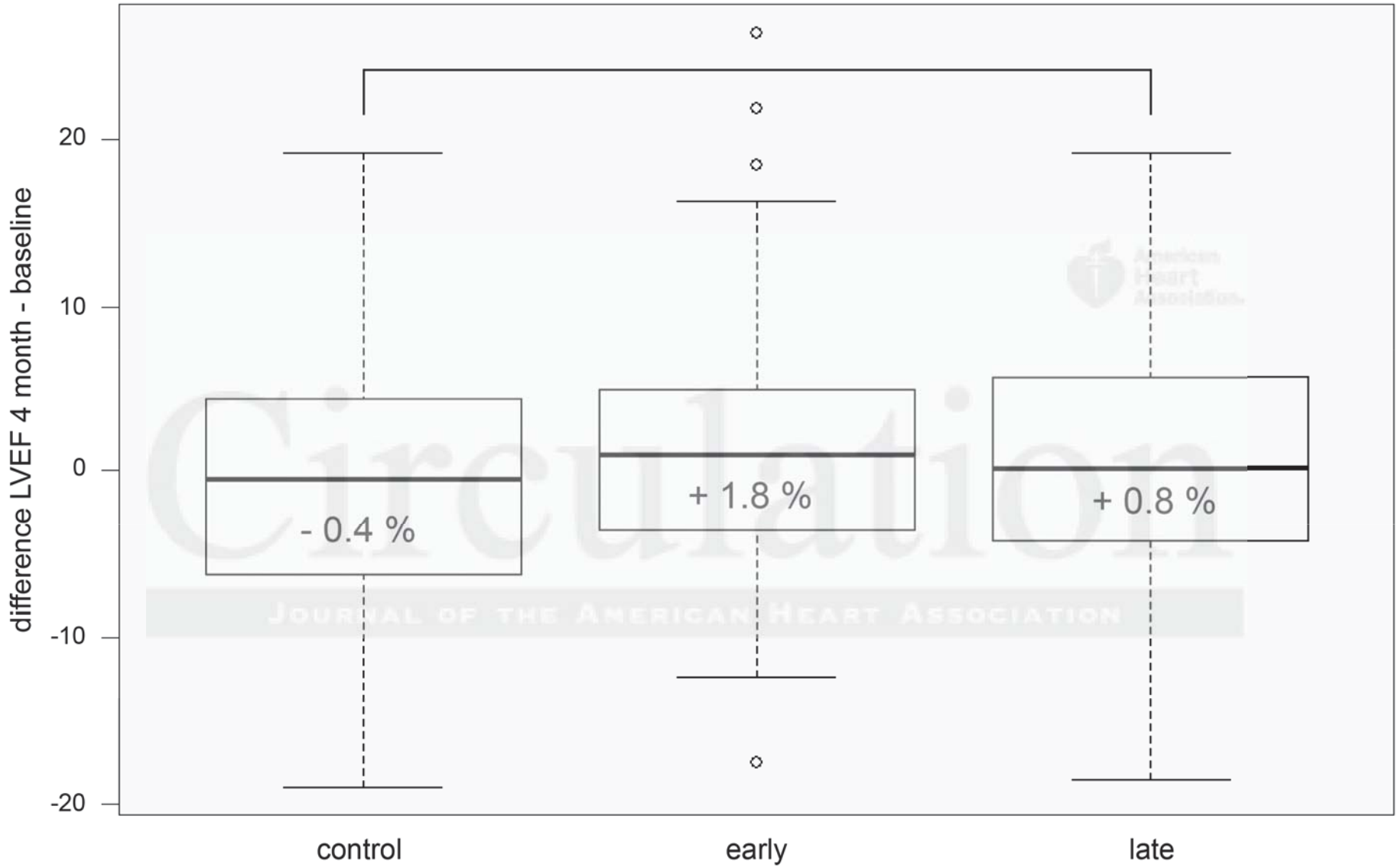
difference LVEF 4 month - baseline
20
10
0
-10
-20
- 0.4 %
+ 1.8 %
+ 0.8 %
control
early
late

Figure 2

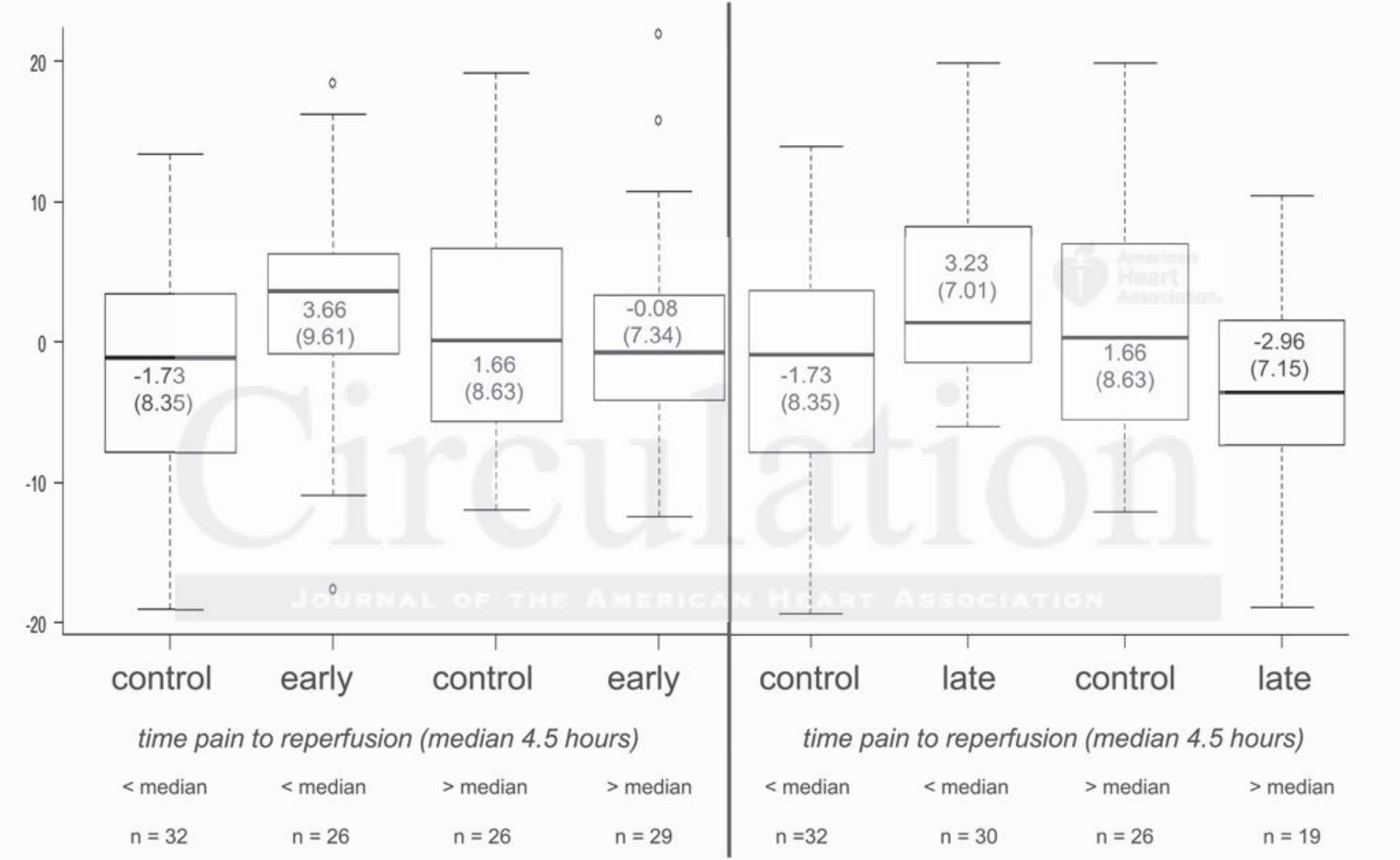
20
10
0
-10
-20
-1.73
(8.35)
3.66
(9.61)
1.66
(8.63)
-0.08
(7.34)
-1.73
(8.35)
3.23
(7.01)
1.66
(8.63)
-2.96
(7.15)
control
early
control
early
control
late
control
late
time pain to reperfusion (median 4.5 hours)
time pain to reperfusion (median 4.5 hours)
< median
< median
> median
> median
< median
< median
> median
> median
n = 32
n = 26
n = 26
n = 29
n =32
n = 30
n = 26
n = 19

Figure 3

SUPPLEMENTAL MATERIAL

Table S1: BM-MNC characterization II

	Median (IQR)	n
Lymphocytes (% of total nucleated cells)§	46 (17)	116
Monocytes (% of total nucleated cells)§	8 (3)	105
CFC (colonies/10^6 cells)*	4050 (2638)	19
CFU-Hill (colonies/10^6 cells)**	11 (11)	6
CFU-F (colonies/10^6 cells)***	12 (11)	15

IQR: interquartile range; §differential cell counting by automated hematology cell analyzer; *CFC assay for the evaluation of hemopoietic cell precursors; **CFU-Hill assay for the evaluation of angiogenic potential; ***CFU-F for the evaluation of mesenchymal precursors.

Methods (not described in the main article):
Lymphocytes, Monocytes and Granulocytes were determined by Differential cell count using automated hematology cell analyzer (ABX Micros 60, Horiba medical).
Colony-Forming Cell (CFC) assay was performed plating cells in Methocult® (StemCell Technologies); after 14 days, plates were microscopically scored for the presence of hematopoietic colonies. For Colony-Forming Unit-Hill (CFU-Hill) assay, cells were suspended in Complete CFU-Hill medium (StemCell Technologies), then seeded in fibronectin coated 6 well plates; after 2 days, non adherent cell were collected, transferred in fibronectin coated 24 well plates and incubated for further 5 days; the wells were then fixed, stained and scored for the presence of colonies. For the Colony-Forming Unit-Fibroblast (CFU-F) assay, cells were plated in Mesencult®-XF (StemCell Technologies); after 14 days, the dishes were fixed, stained and scored for the presence of colonies.

Table S2: Analyses of covariance (ANCOVA) for LVEF, adjusted for baseline LVEF and for BM-MNC related variables

Variable	Median (IQR)	n	Estimate	95% CI	p
Bone marrow aspirate (ml)	70 (15)	105	0.03	-0.08 to 0.13	0.63
MNC (10^6 cells)	153 (119)	105	-0.003	-0.02 to 0.01	0.71
Cell Viability (%)	93.5 (6)	105	-0.04	-0.31 to 0.23	0.79
% CD34+ cells	1.12 (0.83)	105	-0.94	-2.63 to 0.76	0.27
Total CD34+ cells (10^6 cells)	1.55 (1.93)	105	-0.28	-0.91 to 0.35	0.37
% CD34+/CD133+ cells	0.84 (0.87)	105	0.02	-0.03 to 0.67	0.47
CD34+/CD133+ cells (10^6 cells)	0.94 (1.67)	105	-0.07	-0.80 to 0.65	0.84
% Invasion	29 (19)	55	0.08	-0.08 to 0.23	0.32
Invasion index	49.3 (25.5)	55	0.05	-0.07 to 0.16	0.42

Explanation: For each additional ml of bone marrow aspirate and adjusted for baseline LVEF, LVEF at 4 months is on average changed by 0.03% with 95% confidence interval [-0.08 to 0.13] and p-value 0.63, based on 105 complete observations.

% Invasion: percentage of total nucleated cells showing invasion capacity; MNC: Total nucleated cells

Table S3: NYHA and CCS Class at 4 months follow-up

NYHA class	Control	Early	Late	p
1	52 (85.2%)	46 (75.4%)	43 (76.8%)	
2	6 (9.8%)	13 (21.3%)	12 (21.4%)	0.23 *
3	2 (3.3%)	2 (3.3%)	1 (1.8%)	0.24 ‡
4	1 (1.6%)	0	0	
CCS class	**Control**	**Early**	**Late**	**p**
1	59 (96.7%)	56 (91.8%)	53 (94.6%)	
2	1 (1.6%)	3 (4.9%)	3 (5.4%)	0.51 *
3	1 (1.6%)	2 (3.3%)	0	0.35 ‡
4	0	0	0	

NYHA: New York Heart association; CCS: Canadian Cardiac society

*: p value control vs. early; ‡: p value control vs. late